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# Statins in Unconventional Secretion of Insulin-Degrading Enzyme and Degradation of the Amyloid- $\beta$ Peptide

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## Key Words

Alzheimer's disease • Metalloproteinase • Unconventional secretion • Insulin-degrading enzyme • SlyX motif • Statins

## Abstract

Population-based studies demonstrated that statins might decrease the risk of developing Alzheimer's disease (AD). Statins inhibit the 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase and thereby de novo synthesis of cholesterol. Cell culture and animal studies indicated that cholesterol affects the proteolytic processing of the amyloid precursor protein and the generation of amyloid- $\beta$  (A $\beta$ ). Recently, we have demonstrated that statins can also stimulate the degradation of A $\beta$ . The statin-induced clearance of A $\beta$  could be attributed to increased release of the insulin-degrading enzyme (IDE) via an exosome-related unconventional secretory pathway. Interestingly, this statin-induced secretion of exosome-associated IDE was independent of cellular cholesterol concentrations, but rather caused by impairment of isoprenoid biosynthesis and protein prenylation. We further identified a new hexapeptide sequence in the C-terminal region of IDE, named the SlyX motif that is critically involved in IDE secretion. Taken these findings together, the increased clearance of A $\beta$  by stimulated secretion of IDE might contribute to the protective effects of statins against AD.

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## Statins and Alzheimer's Disease

Epidemiological studies indicate that statins exert a beneficial effect against the development of Alzheimer's disease (AD) [1–3]. While the underlying mechanisms are unclear, cellular and animal studies showed that changes in membrane cholesterol affect the proteolytic generation of amyloid- $\beta$  (A $\beta$ ) from the amyloid precursor protein. A $\beta$  is a major component of extracellular plaques in AD patients and exerts neurotoxicity [4–6]. Most studies indicate that lowering cholesterol levels decreases the secretion of A $\beta$ . However, moderate reduction of cholesterol could also promote the generation of A $\beta$  in neuronal cells [7]. The cholesterol-dependent generation of A $\beta$  could involve changes in the distribution of certain secretases and the amyloid precursor protein (APP) in distinct subcellular compartments and membrane microdomains/lipid rafts [7–10].

Statins inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase (HMGCR), a rate-controlling enzyme in the mevalonate pathway that not only produces cholesterol, but also isoprenoids [11]. Statins affect various (patho)physiological processes, like atherosclerosis, vascular inflammation, platelet activation, blood coagulation, and smooth muscle cell proliferation [12, 13]. However, the relative contribution of cholesterol and isoprenoid metabolism to these effects is largely unknown.

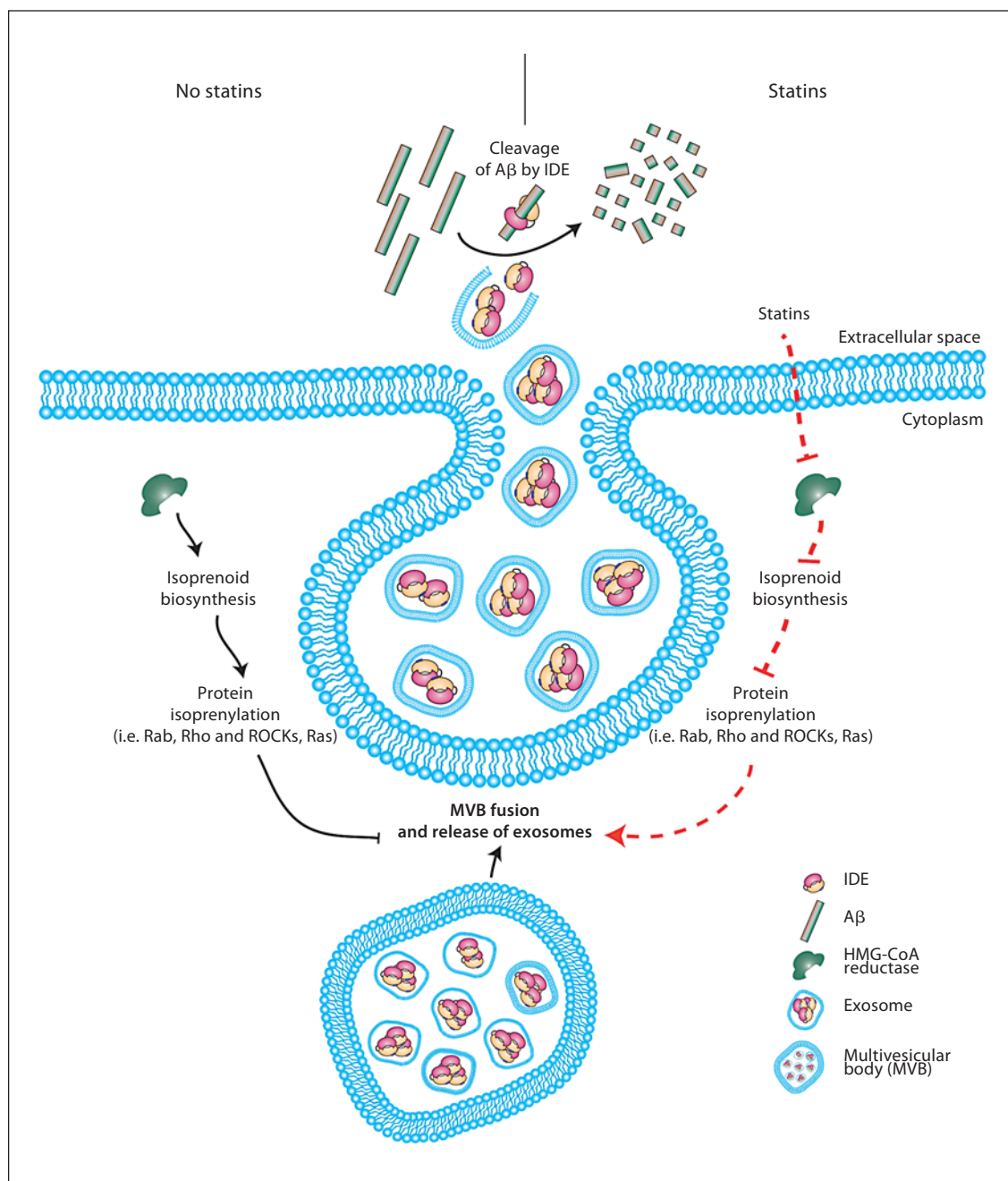
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**Fig. 1.** Suggested mechanism of statin-stimulated Aβ degradation by release of exosome-associated IDE. Exosomes correspond to intraluminal vesicles of endosomal multivesicular bodies released upon fusion with the plasma membrane. Isoprenoids are synthesized in the mevalonate pathway. HMGCR is the rate-controlling enzyme in the biosynthesis of both cholesterol and isoprenoids, thereby regulating the isoprenylation of different proteins like

Ras, Rab, Rho and ROCKs. Statins inhibit HMGCR, and thus also decrease protein prenylation. The decrease in protein prenylation stimulates the fusion of multivesicular bodies and release of exosomes containing IDE. IDE might be released upon breakage of exosomes allowing the degradation of extracellular Aβ (see text for details). MVBs = Multivesicular bodies.

## Unconventional Release of Insulin-Degrading Enzyme Involves Exosomes

Recently, we demonstrated that statins induce the release of the insulin-degrading enzyme (IDE) in association with exosomes from microglia cells and thereby promote the clearance of extracellular A $\beta$  [14] (fig. 1). IDE is a Zn<sup>2+</sup> metalloproteinase and degrades insulin, but also a number of additional peptides, including A $\beta$ .

While mainly localized in the cytosol, IDE can also be released from cells and found in extracellular fluids and conditioned media of cultured cells [15–17]. It lacks a classical signal sequence that targets it to the conventional secretory pathway. By utilizing bioinformatical and biochemical approaches, we have recently identified a novel motif within the inactive protease domain III of IDE, which we called the SlyX motif [18]. This hexapeptide motif ('EKPPHY') shares 100% homology with an amino acid sequence in the C terminus of the bacterial SlyX protein. Its deletion significantly reduces the secretion of IDE from cultured cells. In turn, fusion of the SlyX motif to GFP, which was expressed in the cytosol, improved its secretion into the medium. Notably, the SlyX motif is also found in other proteins, like heparan sulfate proteoglycans or phospholipid scramblase 1 that undergo unconventional secretion pathways.

The unconventional secretion of proteins could be mediated via exosomes, small 40- to 100-nm cup-shaped vesicles found in extracellular fluids like blood, urine, and cerebrospinal fluid [19, 20]. Exosomes may modulate several biological processes, including inflammation, cell differentiation and proliferation [20–22]. Cell biological studies indicate that exosomes originate from intraluminal

vesicles of multivesicular bodies upon fusion with the cellular plasma membrane [20]. They are enriched in cholesterol, sphingolipids and ceramide, which are also present in lipid rafts and further contain certain proteins like Rab GTPases, flotillin, integrins and tetraspanins [20]. However, little is known about the molecular machinery involved in the regulation of exosome secretion. Only recently has it been shown that some Rab proteins, like Rab35, are involved in exosome release [23]. In addition, other prenylated proteins involved in vesicular trafficking, like Ras, Rho and ROCK proteins, might also contribute to this pathway. Interestingly our data indicate that the statin-induced secretion of exosomes and the clearance of A $\beta$  are due to the inhibition of protein prenylation [14]. Since Rab proteins are regulated by isoprenylation [24], it will be interesting to analyze whether statins affect the isoprenylation of certain Rab or other proteins, that are involved in unconventional secretion.

## Conclusions

The recent identification of an amino acid-based signal in IDE and its unconventional secretion via exosomes helped to understand the mechanisms underlying the extracellular occurrence of this enzyme. As statins strongly stimulate exosome release, the beneficial effects of these drugs against AD pathogenesis could involve stimulation of extracellular A $\beta$  degradation. It will now be interesting to further characterize the identified pathway as well as to specifically stimulate secretion of endogenous IDE in therapeutic and/or preventive strategies against AD.

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